



Discovery of frogs of the *Stumpffia hara* species group (Microhylidae, Cophylinae) on Montagne d'Ambre in northern Madagascar, with description of a new species

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http://zoobank.org/EDA7BBEE-CF8F-41E5-9FFE-68B468AB8A1F

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Academic editor: Alexander Haas ♦ Received 11 October 2021 ♦ Accepted 1 February 2022 ♦ Published 23 February 2022

Abstract

The stump-toed frogs of the Madagascar-endemic genus Stumpffia are mostly diminutive in size, but there is one group of comparatively large frogs within the genus, which we herein refer to as the Stumpffia hara species group. Each of the four known members of this species group is endemic to a single location of deciduous dry forest with exposed karstic limestone rock. Here, we report on the discovery of members of this species group on Montagne d'Ambre, a rainforest-covered extinct volcano in the North of Madagascar that has a rich Stumpffia fauna but has been thought to lack members of the S. hara species group until now. We found two members of the species group, one at the peak, and one in transitional and dry deciduous forest on the west and northern slopes of the mountain. The high-elevation species is new to science, and we here describe it as Stumpffia bishopi bishopi

Key Words

New species, Stumpffia bishopi sp. nov., phylogeny, montane rainforest, body size, bioacoustics

Introduction

Stumpffia is a diverse genus of narrow-mouthed frogs (family Microhylidae) in the subfamily Cophylinae, endemic to Madagascar. The genus includes some of the smallest vertebrates in the world (Vieites et al. 2009, Köhler et al. 2010, Klages et al. 2013, Perl et al. 2014, Scherz et al. 2016), including species under 9 mm adult

snout—vent length. They are mainly distributed in northern and northeastern Madagascar. A major revision of the taxonomic status of these frogs was recently undertaken by Rakotoarison et al. (2017) and currently, the genus has 42 described species (AmphibiaWeb 2021).

Within *Stumpffia*, one clade consists of four comparatively large species, *S. be*, *S. hara*, *S. megsoni*, and *S. staffordi*, all of which were described in 2010 (Köhler

et al. 2010, Rakotoarison et al. 2017). These species are all 21–28 mm in adult body size (snout–vent length), and all possess fully developed fingers and toes with enlarged discs. They are thus rather morphologically distinctive within the genus, which otherwise consists mostly of small species, usually with some degree of digital reduction and rarely having enlarged terminal discs on fingers or toes (Rakotoarison et al. 2017). All four species are associated with limestone caves or boulders, often in areas of dry deciduous forest, across northern Madagascar and are not known to be found within rainforest. Given their morphological distinction and close relationships, we here refer to this rather unusual clade as the *Stumpffia hara* species group (corresponding to clade A3 in the phylogenetic analysis of Rakotoarison et al. 2017).

Montagne d'Ambre National Park, a protected area in the North of Madagascar, was until now known to be home to four species of *Stumpffia*, *S. angeluci*, *S. huwei*, *S. madagascariensis*, and *S. maledicta*, each occurring within a specific elevation range on the rainforest-covered mountain (Rakotoarison et al. 2017), analogous to the distribution of *Stumpffia* species in Marojejy National Park in northeastern Madagascar (Rakotoarison et al. 2019). No member of the *S. hara* species group has previously been recorded from Montagne d'Ambre. During fieldwork in 2017–2018, we encountered two members of this group, one at the peak of the mountain, and the other from low-elevation forests. Here we report on these species, one of which is new to science.

Materials and methods

Specimens were collected at night or by day by searching in the leaf litter and among the rocks, partly guided by the calling of males. The search was conducted using torches and headlamps at night. Videos and in-situ photographs of frogs were taken with an Olympus TG-4 camera under headlamp illumination.

Advertisement calls were recorded in the field on a Tascam DR-05 hand-held digital recorder or Marantz PMD-661 MkII with a Sennheiser K6+ME66 super-cardioid microphone and saved as uncompressed files at a sampling rate of 44.1 kHz. Recordings were sampled at 22.05 kHz and 16-bit resolution and computer-analysed in CoolEdit Pro version 2000. Frequency information was obtained through Fast Fourier Transformation (FFT, width 1024 points); the audiospectrograms were obtained at Hanning window function with 256 bands resolution using the seewave package (Sueur et al. 2008) in R (R Core Team 2014). Amplitude in spectrograms is colour-coded with relative dB values referring to the loudest sound in the recording. Temporal measurements are given in milliseconds (ms) or seconds (s), as range, with mean \pm standard deviation in parentheses. We follow the call-centred terminology of Köhler et al. (2017). Calls are deposited on FigShare (https://doi.org/10.6084/ m9.figshare.17706026).

Specimens were euthanized in tricaine mesylate (MS-222) solution, fixed in 90% ethanol, and preserved in 70% ethanol. Before fixation, tissue samples were taken and stored in pure ethanol for molecular analyses. Vouchers were deposited in the Zoological Collection of the Université d'Antananarivo, Mention Zoologie et Biodiversité Animale, Antananarivo, Madagascar (UADBA) and the Zoologische Staatssammlung München, Munich, Germany (ZSM). A specimen of the new species described here that was originally deposited in the ZSM has been repatriated to UADBA; its ex-ZSM number is also given here for completion. Field number abbreviations refer to the zoological and tissue collections of M.D. Scherz (MSZC and MSTIS) and F. Glaw (FGZC).

The following morphological measurements on preserved specimens were taken by AR with digital callipers to the nearest 0.1 mm: snout–vent length (SVL), maximum head width (HW), head length, measured from the rictus to the anterior extent of the upper jaw (HL), horizontal tympanum diameter (**TD**), horizontal eye diameter (**ED**), eye-nostril distance (END), nostril-snout tip distance (NSD), nostril–nostril distance (NND), forelimb length, from the body wall to the tip of the longest finger with the forelimb straightened (FORL), hand length, from the base of the inner metacarpal tubercle to the tip of the longest finger (HAL), hindlimb length, from the cloaca to the tip of the longest toe with the hindlimb straightened (HIL), foot length including tarsus, from the base of the tarsus to the tip of the longest toe (**FOTL**), foot length, from the base of the inner metatarsal tubercle to the tip of the longest toe (FOL), and tibia length (TIBL). Terminology and description scheme follow Rakotoarison et al. (2017).

Genomic DNA was extracted using a standard salt extraction protocol (Bruford et al. 1992). We amplified and sequenced non-overlapping 3' (full alignment length 593 bp) and 5' (full alignment length 653 bp) segments of the mitochondrial 16S rRNA marker, as used in previous work on *Stumpffia* (e.g. Rakotoarison et al. 2017, 2019). The 3' 16S rRNA segment was sequenced using the Illumina amplicon approach of Vences et al. (2016). The 5' 16S rRNA segment was sequenced following Rakotoarison et al. (2019), with the primers 16SL3 and 16SAH. Newly attained sequences were submitted to GenBank (OM388513–OM388537).

Sequences were combined with published sequences from GenBank (full list in Suppl. material 2: Table S1) and aligned with the MUSCLE algorithm implemented in ALIVIEW v1.27 (Larsson 2014). Taxon sampling for the complete tree included at least one individual of all described *Stumpffia* species (42; AmphibiaWeb 2021). *Anilany helenae* (the putative sister genus of *Stumpffia*; Scherz et al. 2016) was used as an outgroup. The alignments of the different 16S fragments were quality-checked by eye and manually adjusted, and then concatenated. The final alignment was 1246 bp and contained 70 terminals. The optimal model was determined to be TIM2+I+G in JMODELTEST v2.1.10 (Darriba et al. 2012) based on the Akaike information criterion. A phylogenetic analysis

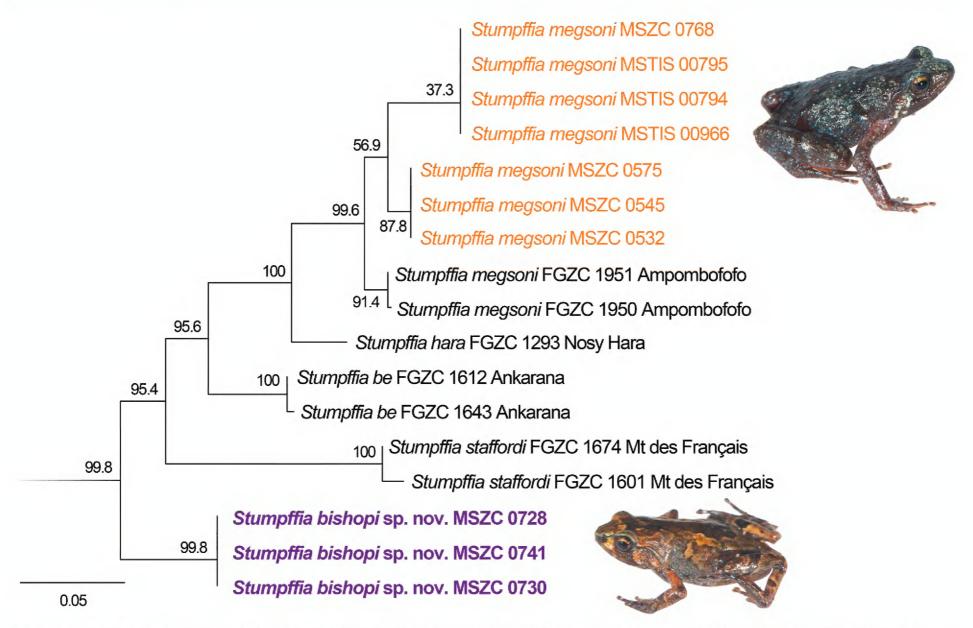


Figure 1. Maximum Likelihood phylogeny of *Stumpffia* species of the *S. hara* species group, based on 1246 bp of the 3' and 5' segments of the 16S rRNA mitochondrial gene. DNA sequences highlighted in colour were newly obtained for this study from Montagne d'Ambre National Park. Numbers at nodes indicate bootstrap support in percent based on 500 bootstrap replicates. All other *Stumpf-fia* species and the outgroup, *Anilany helenae*, are here removed for graphical purposes, and included in Suppl. material 1: Fig. S1.

was conducted under Maximum Likelihood in RAX-ML-NG (Kozlov et al. 2019), with 500 rapid bootstraps. Note that the resulting full tree (Suppl. material 1: Fig. S1) serves as a guide for the identification of the focal taxa of this study, and is not intended to be a robust phylogenetic hypothesis for the genus, for which the data would be far from adequate.

We calculated the uncorrected pairwise distances (p-distances) of the focal taxa using TAXI2, implemented in ITAXOTOOLS (Vences et al. 2021), which uses pairwise gap deletion, based on the 5' fragment of the 16S rRNA gene, because the Illumina-sequenced 3' fragment is shorter than that typically used for p-distance analysis.

This published work and the nomenclatural acts it contains have been registered in ZooBank. The Life Science Identifier (LSID) for this publication is: urn:l-sid:zoobank.org:pub:EDA7BBEE-CF8F-41E5-9FFE-68B468AB8A1F.

Results

Molecular results

The *Stumpffia hara* species group was recovered as monophyletic with strong support in our Maximum Likelihood phylogeny (Fig. 1, Suppl. material 1: Fig. S1), and its topology corresponded with previous work (Köhler

et al. 2010, Scherz et al. 2016, Rakotoarison et al. 2017, Tu et al. 2018). The species we found at the top of the mountain fell at the base the S. hara species group (Fig. 1), and differed from all other members of group by \geq 9.8% (Table 1; and by equally large genetic distances from all other nominal species of *Stumpffia*) in the 5' 16S fragment. The large mitochondrial divergence to any other Stumpffia, and isolated position of the lineage in our phylogenetic reconstruction, as well as the morphological differences to all other species (see below), make it clear that this is a previously unknown species, which we describe in the following. The large *Stumpffia* species found at low elevation in the rainforests of Montagne d'Ambre clustered with specimens of *Stumpffia megsoni* (Fig. 1), differing by 2.5–2.8% in the analysed 5' fragment of the 16S rRNA gene (Table 1). Together, they fell sister to Stumpffia hara with full support, as found in previous work. Below, we provide new data on this species.

Stumpffia bishopi sp. nov.

http://zoobank.org/ADEBEEB7-6801-4C60-B463-9D9BE54F0A56

Remark. This species has not been previously listed as a candidate species in any publication.

Holotype. ZSM 106/2018 (MSZC 0728), adult individual (sex unknown) (Figs 2–3), collected between 2 and 4 December 2017 in high elevation rainforest near Grand Lac

Table 1. Uncorrected pairwise distances among members of the *Stumpffia hara* species group in the 5' segment of the 16S rRNA gene (653 bp).

		1	2	3	4	5	6	7	8	9	10	11
1	Stumpffia be FGZC 1612 GU989211											
2	Stumpffia be FGZC 1643 GU989228	0.002										
3	Stumpffia hara FGZC 1293 GU989210	0.097	0.099									
4	Stumpffia hara FGZC 1294 GU989229	0.098	0.100	0.007								
5	Stumpffia hara FGZC 1295 GU989230	0.093	0.095	0.007	0.007							
6	Stumpffia hara FGZC 1296 GU989231	0.093	0.095	0.007	0.007	0.000						
7	Stumpffia megsoni MSZC 0532	0.130	0.132	0.074	0.078	0.078	0.078					
8	Stumpffia megsoni FGZC 1950 MF768206	0.125	0.127	0.073	0.077	0.077	0.077	0.028				
9	Stumpffia megsoni FGZC 1951 GU989213	0.121	0.122	0.069	0.073	0.073	0.073	0.025	0.002			
10	Stumpffia bishopi sp. nov. MSZC 0741	0.098	0.100	0.119	0.123	0.119	0.119	0.132	0.130	0.127		
11	Stumpffia staffordi FGZC 1601 MF768232	0.100	0.103	0.085	0.085	0.088	0.088	0.100	0.101	0.101	0.100	
12	Stumpffia staffordi FGZC 1674 GU989212	0.119	0.121	0.114	0.119	0.119	0.119	0.145	0.151	0.147	0.129	0.003

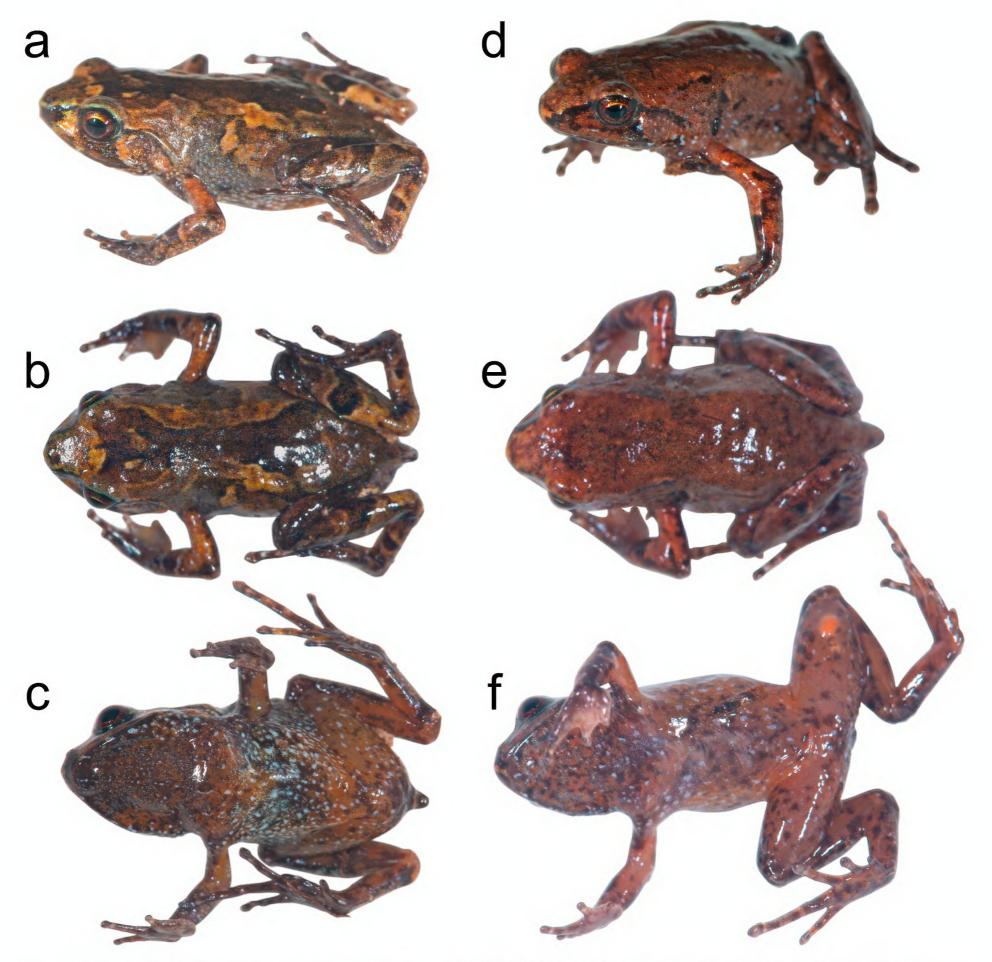


Figure 2. *Stumpffia bishopi* sp. nov. in life **a–c.** Holotype ZSM 106/2018 in **a.** Dorsolateral view; **b.** Dorsal view; **c.** Ventral view; **d–f.** Paratype UADBA 60224 (ex-ZSM 108/2018) in **d.** Anterodorsolateral view; **e.** Dorsal view; **f.** Ventral view. The orange spot on the left ventral thigh in (**f**) is probably a trombiculid mite. Not to scale.



Figure 3. Specimens of *Stumpffia bishopi* sp. nov. in preservative in dorsal (above) and ventral (below) view, and a hand of each in ventral view. Note the difference in first finger shape in ZSM 107/2018 compared to the other two specimens. Photographs by M. Franzen. Not to scale.

on Montagne d'Ambre (12.5970°S, 049.1573°E, ca 1400 m a.s.l.), Diana Region, northern Madagascar, by A. Rakotoarison, A. Razafimanantsoa, J.H. Razafindraibe, M.D. Scherz, O. Randriamalala, R. Tiavina, and S.M. Rasolonjatovo.

Paratypes. ZSM 107/2018 (MSZC 0730), an adult male, with the same collection data as the holotype but collected at 12.5995°S, 049.1592°E, ca 1330 m a.s.l. (Fig. 3); and UADBA-A 60224 (ex-ZSM 108/2018; MSZC 0741), an unsexed adult with the same collecting data as the holotype, but collected at 12.5965°S, 049.1521°E, ca 1480 m a.s.l. (Figs 2, 3).

Diagnosis. A moderately small species of *Stumpffia* from high elevation of Montagne d'Ambre in northern Madagascar. It is assigned to the *Stumpffia hara* species group on the basis of its molecular phylogenetic affinities. The new species is diagnosed by the unique combination of the following characters: (1) Small-sized species (SVL 14.2–16.6 mm); (2) manus with four fingers (first finger slightly reduced in length) and pes with five toes (first toe not reduced in length); (3) enlarged inner metacarpal tubercle; (4) terminal phalanges of fingers without enlarged discs, those of toes with very slightly enlarged discs; (5)

relative hand length HAL/SVL 0.38; (6) relative foot length FOTL/SVL 0.62; (7) dorsum smooth; (8) supratympanic fold distinct; (9) colouration in life dorsally various shades of brown, ventrally with white and black flecks on a taupe to burnt orange background.

Stumpffia bishopi sp. nov. can be distinguished from S. analamaina, S. madagascariensis, S. larinki, S. yanniki, S. tridactyla, S. contumelia, S. obscoena, S. davidattenboroughi, S. betampona, S. dolchi, S. froschaueri, S. makira, S. pygmaea, and S. spandei by larger body size (> 14 mm vs < 14 mm); from S. miery, S. davidattenboroughi, S. tridactyla, S. contumelia, S. tetradactyla, S. makira, S. obscoena, S. betampona, S. dolchi, S. spandei, S. garraffoi, and S. yanniki by a lower degree of digital reduction with the first finger slightly reduced (vs greater reduction in first finger and toe; hands and feet were figured in Rakotoarison et al. 2017); from S. angeluci, S. maledicta, S. sorata, and S. miovaova by fourth finger slightly longer than second (vs fourth finger subequal in length to second); from S. psologlossa by the absence of dark blackish markings along the flank (vs presence), relatively larger hand length (HAL/SVL 0.38 vs 0.18–0.25),

shorter call duration (127–153 ms vs 791–871 ms) and an unpulsed advertisement call (vs distinctly pulsed); from S. analanjirofo by smooth dorsum (vs moderately tubercular); from S. madagascariensis, S. davidattenboroughi, S. tridactyla, and S. contumelia by the lack of a sharp border between dorsal and lateral colour; from S. edmondsi, S. nigrorubra, and S. pardus by the lack of distinct colouration on the posterior shank; from S. iharana by presence of black spots on the venter (vs absence); from S. grandis by the lack of large white markings on the venter (vs present); from S. huwei by the lack of yellowish colouration in the venter (vs present); from S. madagascariensis, and S. angeluci by a shorter call duration (127– 153 ms vs 179–198 ms); from S. larinki, by longer call interval (4388–6355 ms vs 2143–2289 ms), and by a higher frequency range of advertisement calls (3919–4091 Hz vs 2842–3057 Hz); from S. huwei, S. maledicta, S. kibomena, and S. mamitika by longer call duration (127–153) ms vs 70–124 ms); from S. fusca by relatively larger foot length (FOTL/SVL 0.62 vs 0.72–0.78); from S. kibomena, S. meikeae, S. miovaova, S. nigrorubra, and S. rosei*femoralis* by lack of red colour ventrally or on limbs (vs presence); from S. kibomena by unpulsed advertisement call (vs slightly pulsed).

Stumpffia bishopi sp. nov. may be distinguished from other members of the *S. hara* species group as follows: from *S. hara*, *S. be*, *S. megsoni*, and *S. staffordi* by substantially smaller SVL (14.2–16.6 vs 19.8–27.9 mm); additionally, from *S. be*, *S. hara*, and *S. staffordi* by less expanded terminal discs of fingers and toes; from *S. megsoni* and *S. be* by the absence of bright reddish or orange colouration the limbs and abdomen (vs presence; variable in *S. megsoni*, see below); from *S. hara* by the more pronounced supratympanic fold in life, and dark brown pigmentation on the chin (vs absence of pigmentation in examined specimens).

Description of the holotype. Specimen in good state of preservation, left thigh muscle removed as a tissue sample. Body elongate; head slightly longer than wide, narrower than body; snout slightly pointed in dorsal view, pointed in lateral view; nostrils directed laterally, not protuberant, nearer to tip of snout than to eye; canthus rostralis slightly distinct, straight; loreal region concave, weakly oblique; tympanum distinct, about 52% of eye diameter;

supratympanic fold not recognizable (was distinct in life; Fig. 2); tongue long and narrow, broadening posteriorly, attached anteriorly, not notched; maxillary teeth and vomerine teeth absent; choanae rounded. Forelimb brachium (upper arm) slender, antebrachium (lower arm) robust; subarticular tubercles single, slightly distinct; outer metacarpal tubercle distinct, small; prepollical/inner metacarpal tubercle oval, producing a thickening of the inside of the first finger (Fig. 3); fingers without webbing; relative length of fingers 1 < 2 < 4 < 3, fourth finger slightly longer than second, first finger slightly reduced in length; finger tips not expanded into discs. Hind limbs slender; TIBL 46% of SVL; lateral metatarsalia strongly connected; inner metatarsal tubercle small, oval; outer metatarsal tubercle absent; no webbing between toes; toe tips slightly expanded; relative length of toes 1 < 2 < 5 < 3 < 4; fifth toe shorter than third, no toe reduction; subarticular tubercles distinct, single. Skin on dorsum smooth, without distinct dorsolateral folds. Ventral skin smooth.

In life, the holotype was dark brown in base colour with honey brown markings on the flanks, dorsal and lateral head, and shanks, giving it an interesting woodgrain appearance (Fig. 2). The mid-dorsum had a darker brown marking forming a subtriangular area between the back of the eyes and the dorsum that extended down to the postsacral area. There were distinct crossbands on the thighs and less distinct ones on the shanks. The ventral colour was mottled burnt orange posteriorly, flecked with numerous tiny white and black spots, especially white over the anterior abdomen; brown on the sternal area, and dark brown on the chin. After 3.5 years in preservative, the colouration has darkened considerably (Fig. 3); the dorsum is almost uniformly dark brown with the lightest markings faded to a light grey. The venter is cream flecked with grey, more yellow in tone on the ventral legs. The ventral white flecks are no longer distinct, having merged with the base translucency of the skin.

Variation. For morphometric variation, see Table 2. The shape of the first finger differs among specimens, with ZSM 107/2018 having a pronounced first finger without medial swelling (i.e. no developed prepollex), and the other two individuals having a swollen prepollex and consequently short-looking first finger (Fig. 3). In

Table 2. Morphometric data on frogs of the *Stumpffia hara* species group from Montagne d'Ambre. HT = Holotype, PT = Paratype. For measurement abbreviations, see Materials and methods.

	Type	Sex	SVL	HW	HL	TD	ED	END	NSD	NND	FORL	HAL	HIL	FOTL	FOL	TIBL
Stumpffia bishopi sp. nov.																
ZSM 106/2018 (MSZC 0728)	HT	?	14.9	5.3	5.9	0.9	1.7	1.1	0.7	1.5	8.3	6.5	23.5	10.6	3.4	6.9
UADBA-A 60224 (MSZC 0741)	PT	?	14.2	4.5	4.7	1.5	1.9	1.1	0.6	1.4	8.2	5.8	21.2	10.0	3.4	5.5
ZSM 107/2018 (MSZC 0730)	PT	M	16.6	5.6	5.6	1.1	1.7	1.4	0.8	1.7	9.9	6.8	23.3	11.3	3.3	7.2
Stumpffia megsoni																
ZSM 095/2018 (MSZC 0575)		M	19.8	6.8	6.7	1.1	2.3	1.8	1.0	1.9	13.9	9.6	32.2	14.7	5.9	10.0
ZSM 096/2018 (MSZC 0545)		M	22.1	7.5	6.9	1.2	2.5	1.8	1.2	2.2	15.4	11.4	30.5	14.9	5.5	10.4
ZSM 097/2018 (MSZC 0765)		M	21.7	8.7	7.8	1.5	2.7	1.9	1.4	2.3	15.6	11.4	34.1	16.5	5.6	11.8
ZSM 098/2018 (MSZC 0767)		M	23.0	8.5	7.5	1.3	2.0	1.5	1.2	2.6	15.7	11.4	34.5	15.6	5.8	11.8
ZSM 099/2018 (MSZC 0768)		M	24.2	9.4	7.7	1.8	2.4	1.6	1.2	2.6	15.3	11.9	33.7	16.0	5.8	11.7
ZSM 100/2018 (MSZC 0777)		M	23.0	8.8	7.6	1.2	2.2	1.5	1.3	2.5	15.3	11.5	34.3	15.7	5.7	11.7

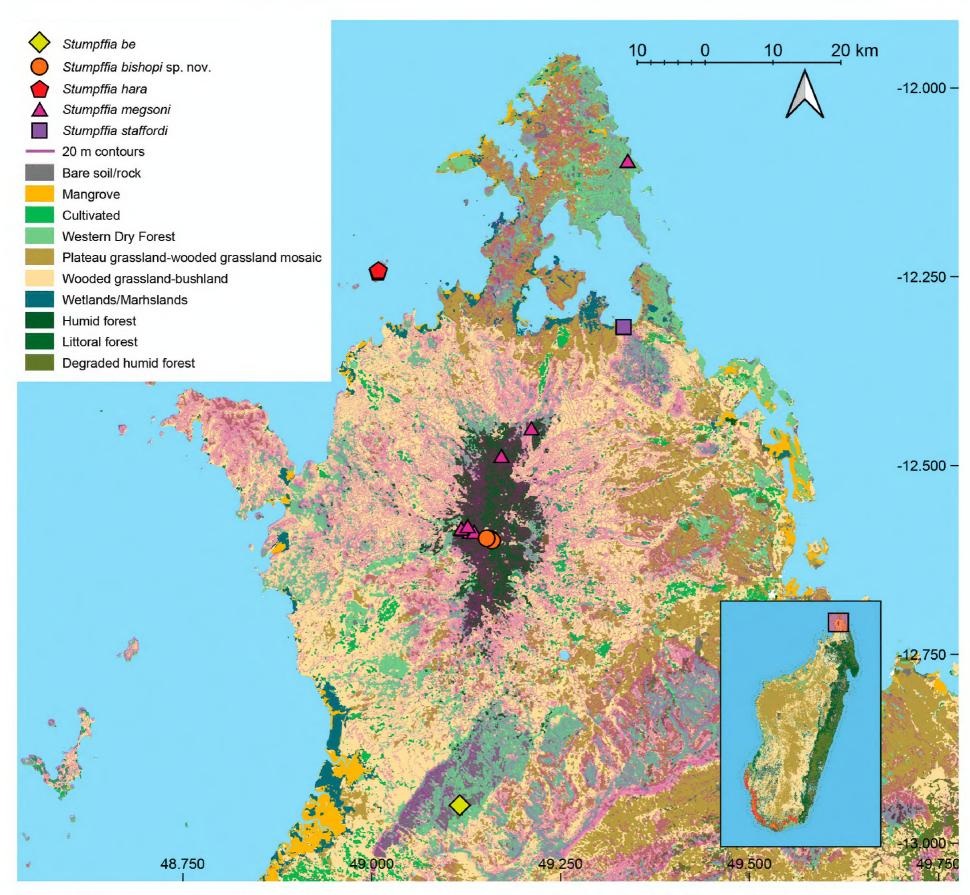


Figure 4. Distribution of the *Stumpffia hara* species group, including the new species *S. bishopi* sp. nov. Vegetation cover map is from the VegMad project by Kew (formerly available at vegmad.org). Elevational contours are extracted from the USGS SRTM 1-Arc Second digital elevation model.

life, the tip of the urostyle protruded distinctly in the specimens we collected (Fig. 2), but it is unclear whether or not this is a diagnostic feature. It diminished in preservative. The colouration of this species is quite variable (see Fig. 2), ranging from the distinct patterning of the holotype to the rather uniform dorsal colouration of the paratype UADBA-A 60224. In all specimens, the inner half of the dorsal hand is consistently light in colour, and the chin is rather dark brown.

Etymology. The species name is a patronym honouring the late Phil Bishop, Professor Emeritus at the University of Otago, who dedicated his life to research on and protection of amphibians. He was an inspirational and incredibly enthusiastic colleague, and we were sorry to lose him far too soon.

Distribution and conservation status. *Stumpffia bishopi* sp. nov. is known only from the Montagne

d'Ambre, northern Madagascar, at high elevations of ca 1330–1480 m above sea level (Fig. 4). The conservation status of the species is in line with other endemics of Montagne d'Ambre: it is known from a single threat-defined location, the protected area of Montagne d'Ambre National Park. Although the extent of occurrence is small enough to qualify for the Critically Endangered category, there are only mild on-going declines to the extent or quality of the habitat, and no known fluctuations in population size or distribution. A change in the protected area's status would jeopardise the survival of the species, however, so we conservatively recommend listing it as Near Threatened.

Natural history. Almost nothing is known of the natural history of this species, except that it lives in the leaf litter of high-elevation rainforest on Montagne d'Ambre. The specimen ZSM 107/2018 (MSZC 0730) was calling from a semi-exposed and slightly elevated

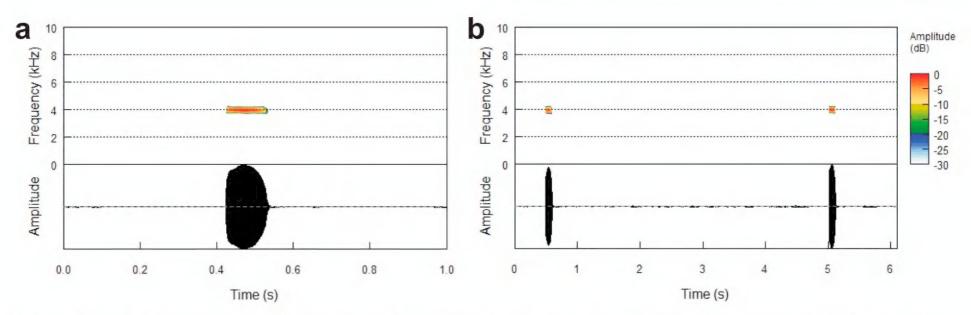


Figure 5. Spectrograms (above) and oscillograms (below) of advertisement calls of *Stumpffia bishopi* sp. nov. from Montagne d'Ambre National Park, recorded from specimen ZSM 107/2018 (MSZC 0730) (Hanning window function at 256 FFT width) **a.** 1 s duration section; **b.** 6 s duration section.

position bordering a path above Grand Lac (12.5995°S, 049.1592°E, ca 1330 m a.s.l.). The left thigh of UADBA 60224 had a small orange spot that is probably a trombiculid mite (Fig. 2; Wohltmann et al. 2007).

Call. Advertisement calls were recorded by S.M. Rasolonjatovo, on 3 December 2017, at 20:25 h, in high elevation rainforest near Grand Lac on Montagne d'Ambre National Park, from the specimen ZSM 107/2018 (MSZC 0730). The individual was sitting on leaf litter. The call consists of a single short tonal note repeated in series at regular intervals (Fig. 5). Numerical parameters are as follows: call duration (= note duration) 127–153 ms (138 \pm 9.3 ms; N = 6), inter-call intervals 4388–6355 ms (5362 \pm 840.5 ms; N = 5), and a dominant frequency at 3919–4091 Hz (3983 \pm 70.6 Hz, N = 6).

New data on *Stumpffia megsoni* Köhler, Vences, D'Cruze & Glaw, 2010

We collected ten specimens of Stumpffia megsoni on Montagne d'Ambre, and took tissue samples from three additional, uncollected individuals. These specimens are surprisingly different in general appearance to the previously known specimens of the species, to which they are however genetically closely related (see above). They are quite large among Stumpffia (SVL 19.8-24.2 mm), within the range of the type specimens of S. megsoni (21.0–22.7 mm; Köhler et al. 2010). They are distinctive in having bright reddish colouration on the portions of the legs that are concealed when in the flexed rest state, as well as the posterior abdomen and thighs (Figs 6, 7). In contrast, the holotype of *S. megsoni* (ZSM 1663/2008) from Ampombofofo had only trace 'orange flecks' on its venter and legs (Köhler et al. 2010). All newly collected individuals had a distinct triangular light green, grey, or brown patch on the dorsal surface of the head, formed by the lines between the eyes and the canthus rostralis. Their finger and toe tips were slightly enlarged, seemingly marginally more so than the type specimens of S. megsoni (Köhler et al. 2010, Rakotoarison et al. 2017).

We encountered this species in two areas on Montagne d'Ambre in 2017–2018: on the west slope between 12.583–12.591°S, 049.118–049.136°E, ca 840–970 m a.s.l., the species was abundant in transitional dry forest that sits on partially exposed volcanic rock with a thin leaf litter layer. Indeed, the species was only found in areas of exposed rock, even in more humid forest slightly higher up the slope, although some individuals were found on dead logs or tree trunks (Fig. 6). This pattern holds for the specimens that were encountered on the north slope, at 12.490°S, 049.172°E (ca 690–710 m a.s.l.) and 12.4537°S, 049.2114°E, ca 480 m a.s.l. There, too, individuals were found on, or very near, exposed volcanic rock. In this northern area, the species seemed substantially less abundant, though we cannot rule out that this apparent difference was due to a temporal effect, as this area was surveyed several weeks after the west slope, and in the lead-up to cyclone Ava, with almost incessant rain.

Despite searching near calling males, we did not succeed in finding any nests, and thus the reproductive mode of the species could not be assessed. To escape capture, individuals dove between the volcanic rocks, which were found to sit loosely atop one another. Although the air temperature in this area was distinctly warmer than that below the uppermost layer of the rocks the temperature, and subterranean air movement could be felt. This may allow exceptional opportunities for behavioural thermoregulation for these frogs.

We were able to make numerous video and audio recordings of calling males (videos available at https://youtu.be/H0JuRQXD_tk). The advertisement call consists of a single short, tonal note without amplitude modulation and with generally limited frequency modulation emitted in series in slow, regular succession. Harmonics were sometimes evident (Fig. 8a, b). Calling specimens were heard during the late afternoon from leaf litter or among rocks. Here, we provide a description of the call of *Stumpffia megsoni* based on field recordings of five specimens (Fig. 8). Temperature data could not be collected alongside recordings due to a faulty thermometer. (1) Calls recorded in the rain on 30th December 2017 at



Figure 6. *Stumpffia megsoni* from Montagne d'Ambre in situ. All individuals were calling adult males. **a.** ZSM 099/2018 (MSZC 0768) calling amongst volcanic rocks; **b.** An uncollected individual calling in an eroded bowl of a volcanic rock; **c.** An uncollected individual calling from a tree truck; **d.** UADBA-A 60225 (MSZC 0764) calling from a fallen log, and **e.** ZSM 096/2018 (MSZC 0545) calling from the leaf litter on the northern slope of Montagne d'Ambre in transitional humid forest. Specimens shown in **a–d** were observed in dry forest on the western slope of Montagne d'Ambre. Video footage of calling individuals **a–d** is available at https://youtu.be/H0JuRQXD_tk.

21:10 h from specimen ZSM 096/2018 (MSZC 0545) at the low camp (Fig. 8a, b) had the following numerical parameters: call duration (= note duration) 104–114 ms (107.5 ± 3.18 ms; N = 10), inter-call intervals 947–1150 ms (1028.1 ± 67.7 ms; N = 10), and a dominant frequency at 3359–3402 Hz (3433.7 ± 70.3 Hz, N = 10); (2) Calls recorded on 8th November 2017 at 18:57 h from specimen ZSM 099/2018 (MSZC 0768) on the west slope (Fig. 8c, d): call duration (= note duration) 122–126 ms (124.8 ± 1.4 ms; N = 9), inter-call intervals 1147–2092 ms (1742.1 ± 258.6 ms; N = 9), and a dominant frequen-

cy at 3229–3316 Hz (3253.2 \pm 38.4 Hz, N = 9); (3) Calls recorded on 8th November 2017 at 19:12 h from specimen ZSM 097/2018 (MSZC 0765) on the west slope: call duration (= note duration) 111–125 ms (118.2 \pm 4.6 ms; N = 10), inter-call intervals 1628–2043 ms (1796.1 \pm 119.8 ms; N = 10), and a dominant frequency at 3316–3445 Hz (3406.3 \pm 47.3 Hz, N = 10); (4) Calls recorded on 8th November 2017 at 21:17 h from specimen UADBA-A 60225 (MSZC 0764) on the west slope (Fig. 8e, f): call duration (= note duration) 141–147 ms (144.5 \pm 1.9 ms; N = 10), inter-call intervals 1736–2412 ms (2088.8 \pm

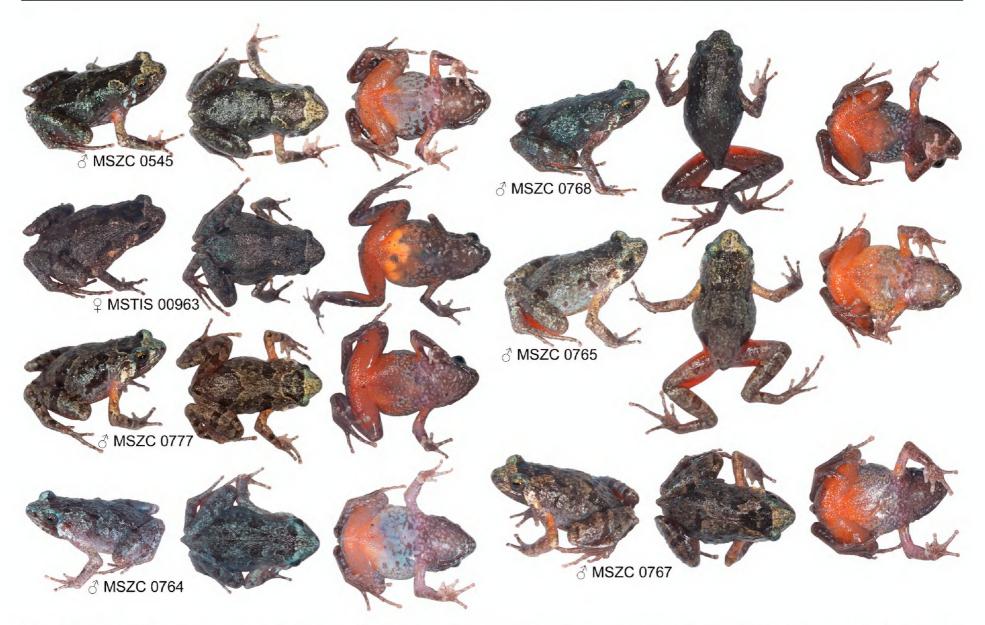


Figure 7. *Stumpffia megsoni* from Montagne d'Ambre in life. All individuals are shown in dorsolateral, dorsal, and ventral view, from left to right. Not to scale.

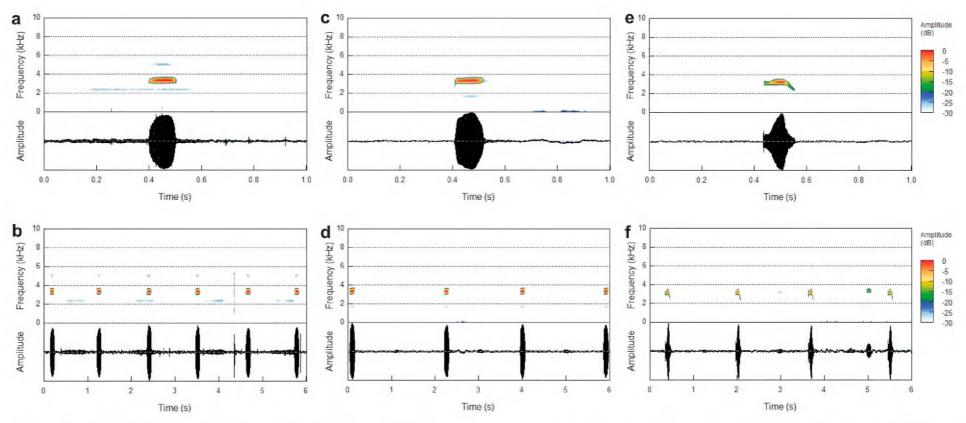


Figure 8. Spectrograms (above) and oscillograms (below) of advertisement calls of *Stumpffia megsoni* from Montagne d'Ambre National Park: **a, b.** 1 s and 6 s duration sections of male ZSM 096/2018 (MSZC 0545); **c, d.** 1 s and 6 s duration sections of male ZSM 097/2018 (MSZC 0765); **e, f.** 1 s and 6 s duration sections of male ZSM 099/2018 (MSZC 0768).

216.6 ms; N = 10), and a dominant frequency at 3273–3445 Hz (3340.9 \pm 54.9 Hz, N = 10); (5) Calls recorded on 9th November 2017 at 18:55 h from specimen ZSM 100/2018 (MSZC 0777) on the west slope: call duration (= note duration) 107–121 ms (113.8 \pm 4.6 ms; N = 10), inter-call intervals 1563–2110 ms (1836.2 \pm 173.2 ms; N = 10), and a dominant frequency at 3186–3445 Hz (3320.1 \pm 79.9 Hz, N = 10).

Some variation was observed in the call interval between some specimens; ZSM 096/2018 had the highest call repetition rate, followed by ZSM 099/2018, and then UADBA-A 60225, ZSM 097/2018, and ZSM 100/2018. UADBA-A 60225 differed from the other individuals by a longer call duration (= note duration). Overall, call duration among all individuals was rather constant, varying between 104–147 ms, whereas inter-call intervals differed

more than two-fold (947–2412 ms). Dominant frequency ranged between 3186–3445 Hz.

These calls differ from those of *S. be* (described by Lattenkamp et al. 2016), by shorter call duration (104–147 vs 170–179 ms), and lower dominant frequency (3186–3445 vs 3899–3928 Hz), but generally the two species have rather similar calls. They are also similar to the calls of *Stumpffia bishopi*, but differ by much shorter inter-call intervals (947–2412 vs 4388–6355 ms), and lower dominant frequency (3186–3445 vs 3919–4091 Hz).

Discussion

The *Stumpffia hara* species group is exceptional in its large body size among a clade of otherwise generally miniaturised frogs. *Stumpffia bishopi* is phylogenetically the earliest diverging member of the group, and is also its smallest species known so far, suggesting a potential gradual increase in size in its members. Köhler et al. (2010) termed this a "reversal" of miniaturisation, and suggested that it may have been associated with changes in ecology, adapting to rockier environments. *Stumpffia bishopi* was not found on rocks or in caves, but in rainforest at high elevation on Montagne d'Ambre. Given its phylogenetic position, forest ecology, and small size with unexpanded finger tips, it indeed supports the hypothesis that ecology, body size, and digit morphology changed together in this clade. Whether they were causally linked, however, will require further study.

Our discovery of two large-bodied *Stumpffia* species on Montagne d'Ambre highlights how incomplete our knowledge of this mountain actually is. Although several herpetological surveys have been carried out in the past 30 years (e.g. Raxworthy and Nussbaum 1994, D'Cruze et al. 2008), each has yielded species new to science (e.g. Raxworthy and Nussbaum 2006, Köhler et al. 2008, Glaw et al. 2009, 2010, D'Cruze et al. 2010, Ratsoavina et al. 2011, Rakotoarison et al. 2015, 2017). Our ~10 week expedition in 2017–2018 yielded several new species, three of which we have described so far (Scherz 2020, Scherz et al. 2020 and the present study), not to mention range extensions for species like Stumpffia megsoni. Although we have often claimed that Montagne d'Ambre has been 'well surveyed' herpetologically, it seems that it still has rather a lot to offer in terms of unknown biodiversity, especially at the upper portion of the massif, which deserves substantial attention (Rasolonjatovo et al. 2020, Scherz et al. 2020 and the present study).

Part of the reason we continue to find new species on Montagne d'Ambre might be pronounced seasonality of the herpetofauna on the mountain, which may be particularly pronounced in the lower and western areas that are drier than the humid rainforest of the peak. Seasonally active species have reduced detectability outside their activity period, and small species are anyway hard to detect. Even these largest *Stumpffia* have a comparatively small body size, and they are very likely to be seasonal in their activity (e.g. Rosa et al. 2012). In 2017, our survey was done

during the rainy season and in areas seldom or never-before visited by herpetologists, and this may have increased our chances of making new and exciting discoveries.

Acknowledgements

We are greatly indebted to A. Razafimanantsoa and J. Miharimanana, for their invaluable assistance in the field. We are also grateful to M. Franzen for providing photographs of the type material of the new species. We thank the Malagasy government (MEDD) for permits to carry out the research (permit no.191/17/MEED/SG/DGF/DSAP/SCB.Re dated 24 July 2017) and the export of samples and voucher specimens (permit no. 032N-EA02/MG18 dated 14 February 2018). This research was supported by Deutsche Forschungsgemeinschaft grants VE 247/13-1 to MV and MDS, and SCHE 2181/1-1 to MDS.

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Supplementary material 1

Figure S1

Authors: Andolalao Rakotoarison, Frank Glaw, Safidy M. Rasolonjatovo, Jary H. Razafindraibe, Miguel Vences, Mark D. Scherz

Data type: Phylogenetic tree

Explanation note: Figure S1. Maximum Likelihood phylogeny of *Stumpffia* based on 1246 bp of the 3' and 5' segments of the 16S rRNA mitochondrial gene. Numbers at nodes indicate bootstrap support based on 500 bootstraps. Note that this tree serves as a guide for the identification of the focal taxa of this study, and is not intended to be a robust phylogenetic hypothesis for the genus, for which the data would be far from adequate. GenBank numbers of the respective sequences are given in Suppl. material 2: Table S1.

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Supplementary material 2

Table S1

Authors: Andolalao Rakotoarison, Frank Glaw, Safidy M. Rasolonjatovo, Jary H. Razafindraibe, Miguel Vences, Mark D. Scherz

Data type: Specimen and accession numbers

Explanation note: Table S1. Specimen and accession numbers of frogs included in phylogenies and p-distance calculations included in this paper.

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